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The accuracy of cell-free fetal DNA based non-invasive prenatal testing in singleton pregnancies: a systematic review and bivariate meta-analysis

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29 **Abstract**

30 **Background.** Cell-free fetal DNA (cffDNA) non-invasive prenatal testing (NIPT) is
31 rapidly expanding and being introduced at varying rates depending on country and
32 condition.

33 **Objectives.** Determine accuracy of cffDNA-based NIPT for all conditions. Evaluate
34 influence of other factors on test performance.

35 **Search strategy.** Medline, Embase, CINAHL, Cochrane Library, 1997-April 2015.

36 **Selection criteria.** Cohort studies reporting cffDNA-based NIPT performance in
37 singleton pregnancies.

38 **Data collection and analysis.** Bivariate or univariate meta-analysis and sub-group
39 analysis performed to explore influence of test type and population risk. .

40 **Main results.** 117 studies included which analysed 18 conditions. Bivariate meta-
41 analysis demonstrated sensitivities and specificities respectively for: fetal sex
42 0.989(95%CI 0.980-0.994) and 0.996(95%CI 0.989-0.998) 11,179 tests; Rhesus D
43 0.993(0.982-0.997) and 0.984(0.964-0.993) 10,290 tests; trisomy 21 0.994(0.983-
44 0.998) and 0.999(0.999-1.00) 148,344 tests; trisomy 18 0.977(0.952-0.989) and
45 0.999(0.998-1.00) 146,940 tests; monosomy X 0.929(0.741-0.984) and 0.999(0.995-
46 0.999) 6,712 tests. Trisomy 13 was analysed by univariate meta-analysis with a
47 summary sensitivity of 0.906(95%CI 0.823-0.958) and specificity of 1.00(95%CI 0.999-
48 0.100) 134,691 tests. False and inconclusive results were poorly reported across all
49 conditions. Test type did affect sensitivity and specificity, but there was no evidence
50 that population risk did.

51 **Conclusions.** Performance of cffDNA-based NIPT is affected by condition under
52 investigation. For fetal sex and Rhesus status NIPT can be considered diagnostic. For
53 trisomy 21, 18 and 13, the lower sensitivity, specificity and disease prevalence
54 combined with the biological influence of confined placental mosaicism designates it a
55 screening test. These factors must be considered when counselling patients and
56 assessing the cost of introduction into routine care.

57 **Systematic review registration.** PROSPERO CRD42014007174

58

59 **Keywords.** cell-free fetal DNA, non-invasive prenatal testing, diagnostic accuracy

60 **Tweetable abstract.** cffDNA NIPT accuracy high, can be diagnostic for fetal sex and

61 Rhesus, but only screening test in aneuploidy

62

63 **Introduction**

64 Non-invasive prenatal testing (NIPT) utilises cell-free fetal DNA (cffDNA) present in
65 maternal plasma and believed to originate from trophoblast. It was first detected by Lo
66 et al. in 1997 (1) and used to note the presence of the Y chromosome to diagnose fetal
67 sex. NIPT can now be used to test for aneuploidy, and single gene disorders such as
68 cystic fibrosis, Huntington's disease or thanatophoric dysplasia (2-6). Its advantage is
69 that it is non-invasive, avoiding the 0.5-1% risk of miscarriage associated with
70 amniocentesis/chorionic villus sampling (7) and allows timely therapeutic intervention in
71 conditions such as congenital adrenal hyperplasia (CAH) (8). cffDNA is cleared from
72 plasma (in hours) following delivery ensuring individuality for each pregnancy (9). Non-
73 invasive prenatal testing also has health economic implications eliminating the need to
74 give all Rhesus negative women anti-D immunoglobulin prophylaxis.

75 NIPT is being introduced into routine antenatal care across the world at differing
76 speeds, largely influenced by technological advances facilitated by the commercial
77 sector. Current guidance in North America and from the International Society for
78 Prenatal Diagnosis advises a positive NIPT for aneuploidy to be confirmed by invasive
79 testing (10-12) due to the low risk of a false positive result secondary to confined
80 placental mosaicism (CPM). Inconclusive results occur in up to 8.1% (10), with a repeat
81 sample being successful in up to 80% participants (13).

82 Several systematic reviews and meta-analyses evaluating test accuracy have been
83 published (14-18). However these have several limitations: i) they evaluate individual
84 conditions (e.g. fetal sex, Rhesus status or aneuploidy) thus not allowing comparison;
85 ii) have a high risk of bias as they include case-control studies; iii) utilise inferior
86 statistical techniques for meta-analysis and iv) include studies with a significant risk of
87 verification bias due to all participants not receiving a reference test (e.g. karyotype).

88 The aim of our paper is to produce the most comprehensive systematic review and
89 meta-analysis of NIPT and address these issues: include only cohort studies to reduce
90 bias (19); perform bivariate meta-analysis where possible and thirdly to encompass all

indications for antenatal use, so as to enable a more uniformed comparison for the use of NIPT in clinical practice. We also aim to assess aspects of test accuracy that might influence how cffDNA is implemented in the clinical pathway e.g. effect of technique on accuracy and evaluation of false positive, false negative and inconclusive results.

Methods

This review was performed according to recommended methods (20-23) and an *a priori* designed and registered protocol (PROSPERO CRD42014007174).

Identification of studies

Medline, Web of Science, Embase, CINAHL and the Cochrane Library databases were searched for relevant articles by FLM. Grey literature and reference lists were hand searched. The search terms used were 'noninvasive', 'non-invasive', 'non invasive', 'prenatal diagnosis', 'cell free fetal DNA' and 'cell-free fetal DNA'. The full search strategy is available as online supplementary material (Appendix S1). The date of publication was limited from 1997 to 13 April 2015. There was no limitation on language.

Study selection

Study selection was performed in duplicate (FLM, RKM) involving screening of titles and abstracts, then reviewing full manuscripts of selected articles. Disagreements in selection were resolved by MDK. Articles were included based on the following criteria:

Population: Women with a singleton pregnancy, any gestation. Populations could include women of varying risk with high-risk women defined as attending for testing due to pre-existing risk factors: a personal or family history of the condition being tested for, high-risk on routine biochemical screening, abnormal ultrasound scan, and/or raised maternal age. Women were considered low-risk if they had none of the above risk factors.

Test: NIPT based on cffDNA in maternal blood, irrespective of condition being examined.

119 *Reference standard:* Studies must have compared all the cffDNA results with either:
120 karyotype results or birth outcome (either blood sample or phenotype) as appropriate in
121 all participants.

122 *Study design:* Cohort studies.

123 *Exclusion criteria:* pre-implantation testing, fetal cell testing, case-control studies, case
124 series with <5 participants.

125 **Data extraction**

126 Data were extracted in duplicate on the relevant 2x2 tables comparing the non-invasive
127 test with the reference test used for definitive diagnosis. Data were also extracted on
128 factors which may affect test accuracy: participant characteristics (e.g. obstetric
129 history); and test characteristics (e.g. cut offs used, test technique [e.g. PCR, MPS,
130 mass spectrometry]). Information regarding false results and inconclusive results was
131 obtained.

132 When a study used similar laboratory protocols on the same blood samples (e.g.
133 different number of replicates performed) only the best results were included. When a
134 study used different laboratory protocols on different blood samples, but the same type
135 of test technique, these samples were grouped together for analysis. If a study sub-
136 divided samples based on population characteristics (e.g. high-risk vs. low-risk for a
137 condition, or 1st trimester vs. 2nd trimester vs. 3rd trimester) these were grouped
138 together for the summary statistics, and analysed as a sub-group where appropriate.

139 **Quality Assessment**

140 The quality of the studies was assessed using the QUADAS-2 tool (24).

141 **Data synthesis**

142 For each study the 2x2 data were used to calculate sensitivity and specificity with 95%
143 confidence intervals. Heterogeneity was explored by assessing the distribution of
144 results in the Forest plots and summary receiver operating characteristic curves
145 (SROC). Summary measures including sensitivities, specificities, diagnostic odds ratio,
146 positive and negative likelihood ratios along with 95% confidence intervals were

147 calculated using bivariate logistic regression model with an unstructured correlation.
148 This model allows for the correlation between sensitivity and specificity from the same
149 study and for the sensitivities and specificities to have different random effects (25).
150 Meta-analysis was performed when there were more than 5 studies per condition using
151 STATA 13 (StataCorp. 2012, College Station, Texas) (see Appendix S2 for more
152 detail). Sub-group analysis and meta-regression was planned *a priori* to assess effects
153 of study level covariates on test accuracy, namely: population characteristics (level of
154 risk for condition where appropriate i.e. not performed in fetal sex or Rhesus D); test
155 technique (e.g. PCR, MPS) and quality aspects according to QUADAS-2. We used
156 sub-group analyses (as opposed to meta-regression) to assess the influence of all
157 categorical covariates due to model convergence difficulties (26).

158

159 **Results**

160 The search revealed 4433 studies for inclusion. After reviewing the full article, 117
161 studies (1, 27-143) were eligible reporting on 18 different conditions, and 472,935 tests
162 (Figure S1). The study characteristics are outlined in Table S1.

163 We were able to produce summary results using the fully unstructured bivariate model
164 for: fetal sex, Rhesus D, trisomy 21, trisomy 18 and monosomy X (Table S2). For
165 trisomy 13, despite a sufficient number of studies (n=15) there was no heterogeneity in
166 specificities across studies so the bivariate model, which takes into account the
167 correlation between the sensitivities and specificities, failed to converge and
168 consequently we fitted a univariate model. Because of this, these results are less
169 methodologically robust. The HSROC curves are presented in Figure S2 and the
170 results from our sub-group analyses in Table S2.

171 There were 5 studies (n=394,130 tests) in which there was differential verification of
172 results, in that some participants had their result confirmed by karyotype and others by
173 phenotype (35, 91, 93, 114, 133). These 5 studies all assessed fetal aneuploidy and
174 utilised NIPT as a screening test in a low-risk population. A sensitivity analysis

175 removing these 5 studies demonstrated no significant effect on the summary results,
176 thus these studies are included in all analyses and Forest plots.
177 The following 12 conditions had insufficient studies for meta-analysis: Rhesus C,
178 Rhesus E, 47XXX, 47XXY, 47XYY, trisomy 16, congenital adrenal hyperplasia,
179 deletion-duplication syndromes, sickle cell anaemia, thalassaemia, human platelet
180 antigen 1a, and KEL 1. The Forest plots of these 12 conditions are presented in Figure
181 S3.

182

183 ***Methodological quality of included studies***

184 This was assessed according to the Quality Assessment tool for Diagnostic Accuracy
185 Studies (QUADAS-2) (24), the results are demonstrated in Figure S4 and further
186 described in Appendix S3.

187

188 ***False results and inconclusive results***

189 Reporting of causes and implications of false positive, false negative and inconclusive
190 results was poor, and varied across all conditions (Table S3). The included studies
191 reported an inconclusive result rate of 0.32-5.3%. This issue was further compounded
192 by a myriad of varying quality control (QC) standards, some studies excluding samples
193 that failed their QC and others implementing no QC steps and therefore reporting some
194 results as false negatives which other studies would have excluded from analysis.
195 Some studies investigated the reasons for their false and inconclusive results and
196 reported these clearly, accounting for all samples. Other studies reported inconclusive
197 results as false negatives or did not report them at all. We describe these results in
198 more detail for each of the conditions investigated.

199

200 ***Results from bivariate meta-analysis***

201

202 ***Fetal Sex***

203 Sixty studies (11,179 tests) evaluated fetal sex and are represented in the Forest plot
204 in Figure 1. Bivariate meta-analysis produced a summary sensitivity of 0.989 (95% CI
205 0.980 to 0.994) and specificity of 0.996 (95% CI 0.989 to 0.998), a positive likelihood
206 ratio of 255 (95% CI 89 to 729) and negative likelihood ratio of 0.011 (95% CI 0.006 to
207 0.019). Other summary measures are in Table S2.

208 No significant effect on sensitivity was found with test technique. However there was a
209 difference in specificity with real-time quantitative PCR 0.999 (95%CI 0.991 to 1.00)
210 performing better than conventional PCR 0.939 (95%CI 0.872 to 0.972). For fetal sex,
211 11/60 studies reported inconclusive results, of these, 5 studies documented an
212 explanation (in order of frequency): assay failure, no reason given, insufficient number
213 of markers present from pre-specified cut-off and low fetal fraction. The commonest
214 reasons given by the authors of the studies for the false results were: no reason given,
215 low fetal fraction (although cffDNA not quantified), low fetal fraction confirmed by
216 authors quantifying cffDNA, possible contamination/DNA degradation/vanishing
217 twin/test failure although not confirmed, and previous male pregnancy, although the
218 latter reason has since been disproven as cell-free fetal DNA is cleared from the
219 maternal circulation hours post-delivery (9).

220

221 ***Rhesus D***

222 Thirty studies (10,290 tests) evaluated fetal Rhesus D status and are represented in
223 Figure 2. Bivariate meta-analysis produced a summary sensitivity of 0.993 (95% CI
224 0.982 to 0.997) and specificity of 0.984 (95% CI 0.964 to 0.993) a positive likelihood
225 ratio of 61 (95% CI 22 to 167) and negative likelihood ratio of 0.007 (95% CI 0.003 to
226 0.186). There was a significant difference between test techniques with real-time
227 quantitative PCR sensitivity: 0.997 (95% CI 0.987 to 0.999) demonstrating a higher
228 sensitivity than conventional PCR 0.924 (95%CI 0.832 to 0.968), although it was not
229 possible to assess if there was a difference in those which utilised mass spectrometry
230 (despite sufficient studies, due to convergence issues as detailed in the discussion),

231 and no difference in specificity was seen (Table S2). For Rhesus D, 13/30 studies
232 reported inconclusive results, of these, 10 studies documented an explanation (in order
233 of frequency): no reason given, RHD gene variant, insufficient number of markers
234 present from pre-specified cut-off, test failure, low fetal fraction. The commonest
235 reasons given for false results were: presumed low fetal fraction (although not
236 quantified by authors), no reason given, presumed RHD gene variant (although not
237 confirmed), confirmed RHD gene variant, test failure, possible contamination/DNA
238 degradation/pipetting error/incorrect neonatal blood testing.

239

240 ***Trisomy 21***

241 Thirty-one studies (148,344 tests) assessed trisomy 21 and are represented in Figure
242 3A. Bivariate meta-analysis produced a summary sensitivity of 0.994 (95% CI 0.983 to
243 0.998) and specificity of 0.999 (95% CI 0.999 to 1.00) a positive likelihood ratio of 1720
244 (95% CI 1111 to 2662) and negative likelihood ratio of 0.006 (95% CI 0.002 to 0.017).
245 Test technique and population risk had no significant effect. For trisomy 21, 14/31
246 studies reported inconclusive results, of these, 7 studies documented an explanation
247 (in order of frequency): assay failure, confirmed low fetal fraction, no reason given,
248 presumed low fetal fraction/inadequate sequencing depth. The commonest reasons
249 given for false results were: confirmed low fetal fraction, confirmed mosaicism, no
250 reason given, test failure, maternal CNV.

251

252 ***Trisomy 18***

253 Twenty-four studies (146,940 tests) assessed trisomy 18 and are represented in Figure
254 3B. Bivariate meta-analysis produced a summary sensitivity of 0.977 (95% CI 0.952 to
255 0.989) and specificity of 0.999 (95% CI 0.998 to 1.00) and a positive likelihood ratio of
256 1569 (95% CI 810 to 3149) and negative likelihood ratio of 0.023 (95% CI 0.011 to
257 0.048). Neither test technique or population risk had a significant effect. For trisomy 18,
258 12/24 studies reported inconclusive results, of these 7 studies documented an

259 explanation (in order of frequency): low fetal fraction, test failure, no reason given,
260 mosaicism. The commonest reasons given for false results were: confirmed low fetal
261 fraction, confirmed mosaicism, presumed low fetal fraction/human error, maternal CNV,
262 no reason given.

263

264 ***Monosomy X***

265 Eight studies (6712 tests) assessed monosomy X and are represented in Figure 3C.
266 Bivariate meta-analysis produced a summary sensitivity of 0.929 (95% CI 0.741 to
267 0.984) and specificity of 0.999 (95% CI 0.995 to 0.999) and a positive likelihood ratio of
268 1337 (95% CI 213 to 8407) and negative likelihood ratio of 0.071 (95% CI 0.017 to
269 0.292). There was no significant difference with test technique. It was not possible to
270 assess the effect of population risk as there were insufficient low-risk studies. For
271 monosomy X, 5/8 studies reported inconclusive results, of these, 3 studies documented
272 an explanation (in order of frequency): low fetal fraction, presumed human error and no
273 reason given. The commonest reasons given for false results were: mosaicism and no
274 reason given.

275

276 The 5 aneuploidy studies which evaluated an unselected obstetric population reported
277 inconclusive results rates of 0.29-5.1% and provided the same reasons for their false
278 and inconclusive results as with the high-risk aneuploidy populations.

279

280 ***Trisomy 13 – univariate meta-analysis***

281 Sixteen studies which equates to 134,691 tests examined trisomy 13, represented in
282 Figure 3D. There was a summary sensitivity of 0.906 (95% CI 0.823 to 0.958) and
283 specificity of 1.00 (95% CI 0.999 to 1.00). The positive likelihood ratio was 453 (95% CI
284 26 to 7864) and negative likelihood ratio was 0.188 (95% CI 0.080 to 0.44039) with a
285 diagnostic odds ratio of 2788 (95% CI 285 to 27252). For trisomy 13, 6/16 studies
286 reported inconclusive results, of these, 4 studies documented an explanation for

287 inconclusive results: low fetal fraction, different fragmentation rate, contamination,
288 assay failure and human error. The only reason given for false results was confirmed
289 low fetal fraction.

290

291 ***Results where meta-analysis not possible***

292 The results for these conditions are presented as Forest plots in S3.

293

294 **Clinical application for NIPT for Down's syndrome screening**

295 Using published data from the National Down Syndrome Cytogenetic Register
296 (NDSCR) 2012 Annual report we have produced a table detailing the estimated
297 outcomes (livebirth rate, invasive test rate, euploid pregnancy loss rate, undiagnosed
298 aneuploidy livebirth rate) from the current standard Down's Syndrome Screening
299 (DSS) i.e. first trimester combined screening pathway (maternal age, nuchal
300 translucency, beta human chorionic gonadotrophin and pregnancy associated plasma
301 protein A) and from a pathway with NIPT as both contingent (i.e. NIPT offered to
302 women with a positive screen after first trimester combined screening) and first line
303 screening for a population of 100,000 women using crude rates (144) (Table S4). We
304 use the prevalence reported by NDSCR¹ (trisomy 21: 2.2 per 1000 women, trisomy 18:
305 0.64 per 1000, trisomy 13 0.26 per 1000). This assumes that standards for the first
306 trimester combined screening are "achievable" as described by Fetal Anomaly
307 Screening Programme (FASP) guidance i.e. for trisomy 21 a detection rate of 85% for
308 a screen positive rate of 2% (145). For NIPT the summary measures are those from
309 our meta-analysis. For the contingent screening model the cut-off for high risk is 1:1000
310 from first trimester combined screening with a detection rate of 96% and false positive
311 rate of 12% (146). This model assumes that all women accept screening when offered
312 as it is not possible to determine yet what the uptake of NIPT would be if offered as a
313 first-line test. It also assumes that all women are required to have an invasive test for
314 karyotyping after a screen positive result from combined or NIPT prior to considering

315 termination of pregnancy, thus the invasive test rates will be higher than in a real-life
316 population. It assumes a 0.5% pregnancy loss rate from invasive testing (146).
317
318 These data demonstrate the influence of disease prevalence on test performance. If we
319 compare combined screening with a 1:150 cut-off (i.e. current NHS practice) with NIPT
320 as a first-line test we can reduce the invasive test rate from 2000 to 319 per 100,000
321 women, the euploid pregnancy loss rate from 9 to 1 per 100,000 and the undiagnosed
322 trisomy 21 live births rate from 32 to 1 per 100,000. If NIPT was used as a contingent
323 screening test for a 1:1000 combined screening cut-off (i.e. as a 2nd test following a
324 positive combined screening result at a 1:1000 cut-off) then these figures are reduced
325 even further compared to combined screening with a 1:150 cut-off: 2000 to 222 per
326 100,000 women invasive test rate; 9 to 0 euploid pregnancy loss rate, although there is
327 less of a reduction in undiagnosed trisomy 21 live birth rate from 32 to 10. If NIPT was
328 used as a contingent screening test for a 1:150 combined screening cut-off then these
329 figures are: 2000 per 100,000 women invasive test rate; 0 euploid pregnancy loss and
330 34 undiagnosed trisomy 21 livebirth rate. A two stage contingent screening pathway
331 with a 1:1000 cut-off when compared to NIPT as a first line test affords a reduction in
332 false positive results (12 versus 100 per 100,000 women) that are found at the time of
333 NIPT as the prevalence of disease in the population now undergoing NIPT is much
334 higher. This is at the expense of a 10 fold increase in undiagnosed aneuploidy live
335 births (1 versus 10 per 100,000 women) due to the increased number of false
336 negatives at the first stage of screening that do not undergo NIPT. A cut-off of 1:150 at
337 the first stage for the combined test compared to a 1:150 cut-off for NIPT as a
338 contingent screening test has little effect on the number false negatives (33 versus 34),
339 however the invasive test rate is reduced (2000 versus 188 per 100,000 women).

340

341 **Discussion**

342 ***Main findings***

343 Our results demonstrate that for fetal sex and Rhesus D status, cffDNA-based NIPT
344 has a high sensitivity and specificity. For aneuploidies: trisomy 21, and in particular
345 trisomy 18 and 13 we have demonstrated improved accuracy from other recent
346 systematic reviews likely due to technological developments. Importantly we found that
347 false results and inconclusive results were poorly reported across all conditions.

348

349 ***Strengths and limitations***

350 This review was performed according to rigorous methodology with efforts made to
351 reduce bias in participant selection and clinical applicability by excluding case-control
352 studies, performing bivariate meta-analysis and meta-regression analysis and
353 assessing the impact of differential verification (i.e. different reference standards).
354 Bivariate meta-analysis is the recommended approach for the meta- analysis of
355 diagnostic test accuracy studies. This is because a conventional univariate analysis
356 makes assumptions that are known not to be tenable (that the sensitivity and specificity
357 from the same study are independent). However, the bivariate meta-analysis model is
358 a technically difficult model to fit and it is well known that these models might not
359 converge when there are a small number of studies, or when there are zero cells (i.e.
360 sensitivity or specificity close to 100) (26). We observed no indication that other model
361 fits were unstable and so have no reason to be concerned about the statistical validity
362 of the other results. Our review also evaluates more conditions than previously. In
363 addition, our paper has been able to assess the impact of test technique and
364 population risk. We were unable to evaluate the number of samples which failed QC
365 measures as this was reported in varying degrees. When considering the
366 implementation of a new test, information regarding failed tests (147, 148), and
367 inconclusive results is vital. We investigated the reasons for false positive and false
368 negative results within and across studies and attempted to summarise these. This was
369 again hampered by poor reporting with a common reason being low fetal fraction which
370 is difficult to measure accurately and thus has led to variations in approach between

371 studies. It is especially important to consider this further as low fetal fraction has been
372 shown to be associated with trisomy 18 and triploidies.

373 A limitation of this work is that it was not possible to account for the many subtle
374 differences in laboratory techniques such as comparing the different combinations of
375 genetic markers used for each condition; or the myriad of adjustments made to
376 bioinformatics algorithms as these were so varied. This is where the results from the
377 large studies in screening populations are especially important as there is QC across
378 laboratories and standardisation of techniques (35, 91, 93, 114, 133). In the process of
379 publishing this review, the search was re-run from April 2015 - September 2015 in view
380 of the rapid progression in this area. This yielded 78 new citations, of which 11
381 additional papers would be eligible for inclusion (3, 149-158), which comprise 10,191
382 women in total. These studies examine fetal sex (n=436 women), Rhesus D status
383 (n=2965), trisomy 21 (n=6661), trisomy 18 (n=6701), trisomy 13 (n=6495), and
384 monosomy X (n=40), which equate to a small proportion of additional tests, compared
385 to the studies we have already analysed. There is also now one study which
386 investigates thanatophoric dysplasia (n=108), although this cannot be included in a
387 meta-analysis as it is the only study to look at this condition thus far. As the search was
388 under a year old when the publication was accepted we have not included these 11
389 studies in our results. We are confident that if these studies were included they would
390 not impact on our results and conclusions.

391

392 ***Interpretation***

393 It is recognised that there are fewer studies in our meta-analyses for trisomy 13 and
394 monosomy X compared to a previous large meta-analysis (14) but this is due to
395 excluding case-control studies and limiting to singletons. This has led to us reporting
396 higher summary sensitivities and specificities than existing analyses, demonstrating
397 how NIPT is advancing, and supporting the belief that NIPT will be used as the first-line
398 screening test in the future. Our clinical application model has highlighted the

399 importance of low prevalence of disease on the positive predictive value and false
400 positive rate in the case of aneuploidies. Although positive and negative predictive
401 values are useful indicators of test accuracy as they take into account disease
402 prevalence (159), we have not presented these values within this paper due to
403 variation in disease prevalence among included study populations.

404

405 ***Conclusion***

406 This work demonstrates that there is a sufficient body of evidence for the accuracy and
407 reproducibility of cffDNA-based NIPT to allow its introduction into routine clinical
408 practice within the UK, however its role is yet to be decided.

409

410 ***Implications for clinical practice***

411 The findings of this analysis support the use of NIPT as a diagnostic test for fetal sex
412 and Rhesus status due to the nature of these conditions and the populations being
413 tested. For assessment of aneuploidy the test must be considered a “screening test”
414 despite high accuracy due to the low prevalence of disease and influence of biological
415 factors such as CPM. We are aware that the National Screening Committee (NSC) is
416 currently reviewing all the evidence for aneuploidy, and is likely to recommend NIPT as
417 a contingency screening test in the UK (Dr Pranav Pandya, Personal Communication,
418 2015). While for Down’s syndrome screening (DSS) this will ensure access to an
419 accurate, non-invasive test and ensure equity for many more women (i.e. test threshold
420 has less of an impact on offering invasive testing and test can be offered throughout
421 gestation not just in a small first trimester window) this must be balanced with
422 consideration of the important ethical repercussions which need addressing (i.e. a test
423 that can assess for multiple conditions and those with a milder phenotype and also test
424 for conditions within the mother e.g. sex-chromosome anomaly or cancers) (160).
425 There are also counselling implications as access to a non-invasive, highly accurate
426 test still needs careful consideration by parents.

427

428 ***Implications for future research***

429 The authors would recommend that the same rigorous assessment of the evidence and
430 accuracy as we have performed be applied in multiple pregnancies once the evidence
431 base is sufficient.

432 The NIHR funded RAPID study which has used NIPT in an NHS setting for women in
433 whom combined testing gave a risk of $\geq 1:1000$ will soon be published. This study aims
434 to assess the uptake of NIPT and whether the addition of NIPT to the DSS pathway
435 affects the uptake of DSS and invasive testing; a detailed health economic evaluation
436 using a tool developed in conjunction with the UK NSC; optimal ways to deliver
437 education to women and healthcare professionals; and sensitivity and specificity of
438 NIPT for aneuploidy when performed in an NHS regional genetics laboratory. The
439 results from our review indicate the latter (accuracy results from an NHS regional
440 genetics laboratory) will be an important outcome as it will remove the influence of
441 results from the commercial sector and poor reporting. This will allow for improved QC,
442 enable continued assessment on a national basis, and ensure that the cost of NIPT will
443 improve further. Similarly, the conditions for which NIPT will be used are likely to
444 increase; 11 studies which examined single gene mutations and microdeletions could
445 not be included in our meta-analysis due to having fewer than 5 participants; even
446 whilst writing this review larger studies are being reported on these conditions (161).
447 However, an economic evaluation of this first-line screening with NIPT would also need
448 to include maintaining access to a high quality first trimester ultrasound scan including
449 nuchal translucency (NT) assessment, to allow dating, viability, multiple pregnancy,
450 structural anomaly and adnexal assessment, and importantly the assessment of the
451 risk of cardiac anomalies and increased pregnancy loss associated with raised NT.

452

453

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463
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466 contributed to the analysis and data interpretation and amended the manuscript. KH
467 conducted the bivariate meta-analysis and data interpretation and amended the
468 manuscript. SA assisted with data extraction, interpretation of the results and amended
469 the manuscript. MDK conceived, designed and oversaw the work, made final decisions
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477 478 **References**

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924 **Supplementary material legends**

925 **Figure S1** Study selection from initial search

926 **Figure S2** HSROC curves for bivariate analyses

927 **Figure S3** Forest plots of studies bivariate not possible

928 **Figure S4** Bar chart demonstrating quality assessment of included studies from

929 QUADAS-2 risk of bias assessment

930 **Table S1** Characteristics of included studies

931 **Table S2** Bivariate results

932 **Table S3** Reasons for false positives and false negatives and inconclusive results

933 **Table S4** Clinical application for Trisomy 21

934	Appendix S1 Search strategy
935	Appendix S2 Additional statistical methods
936	Appendix S3 Quality assessment results
937	

Figure 1: Forest plot of studies testing fetal sex using cell-free fetal DNA

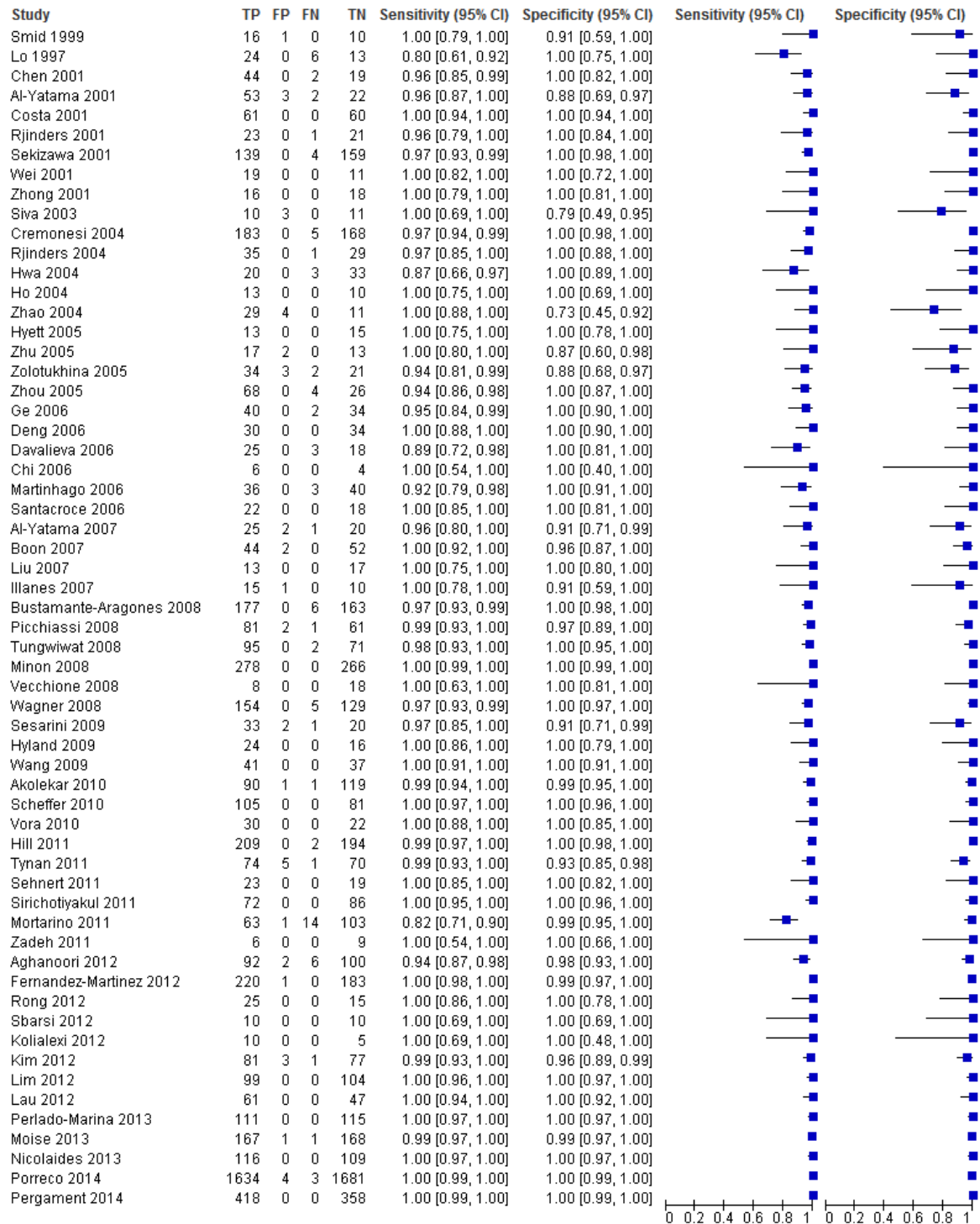


Figure 2: Forest plot of studies testing Rhesus D status using cell-free fetal DNA

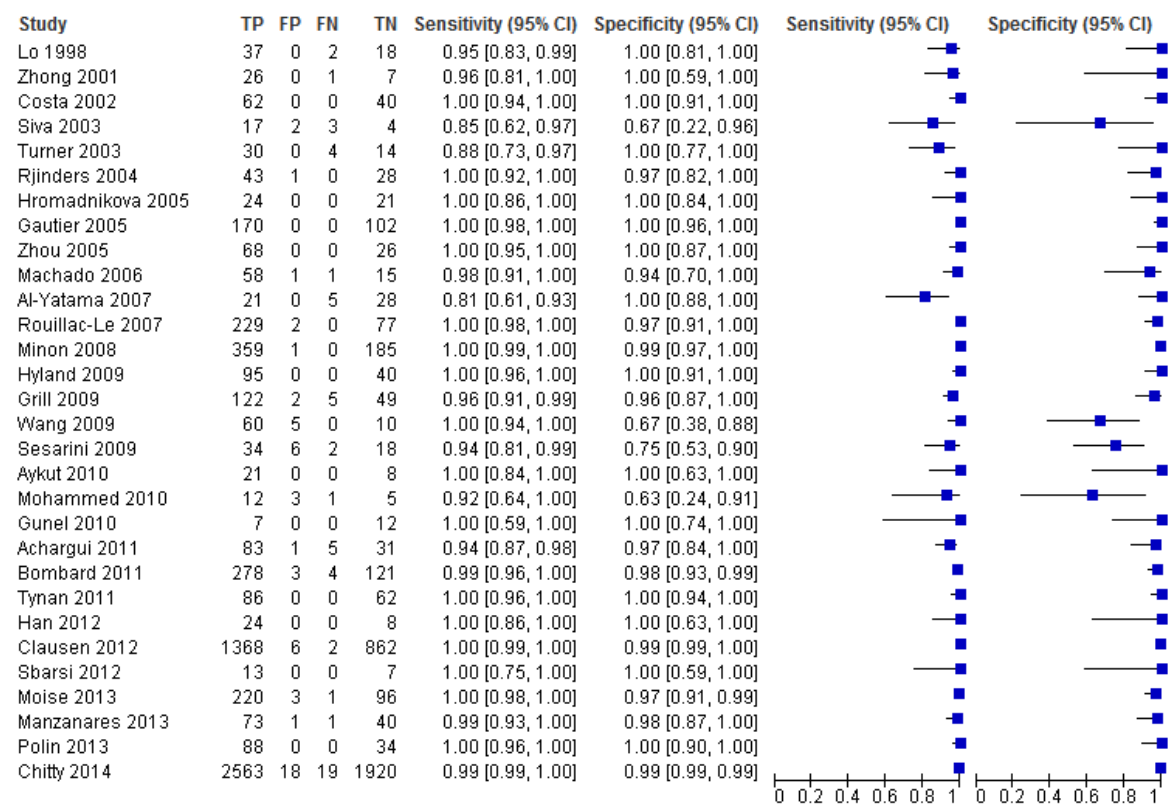


Figure 3A: Forest plot of studies testing Trisomy 21 using cell-free fetal DNA

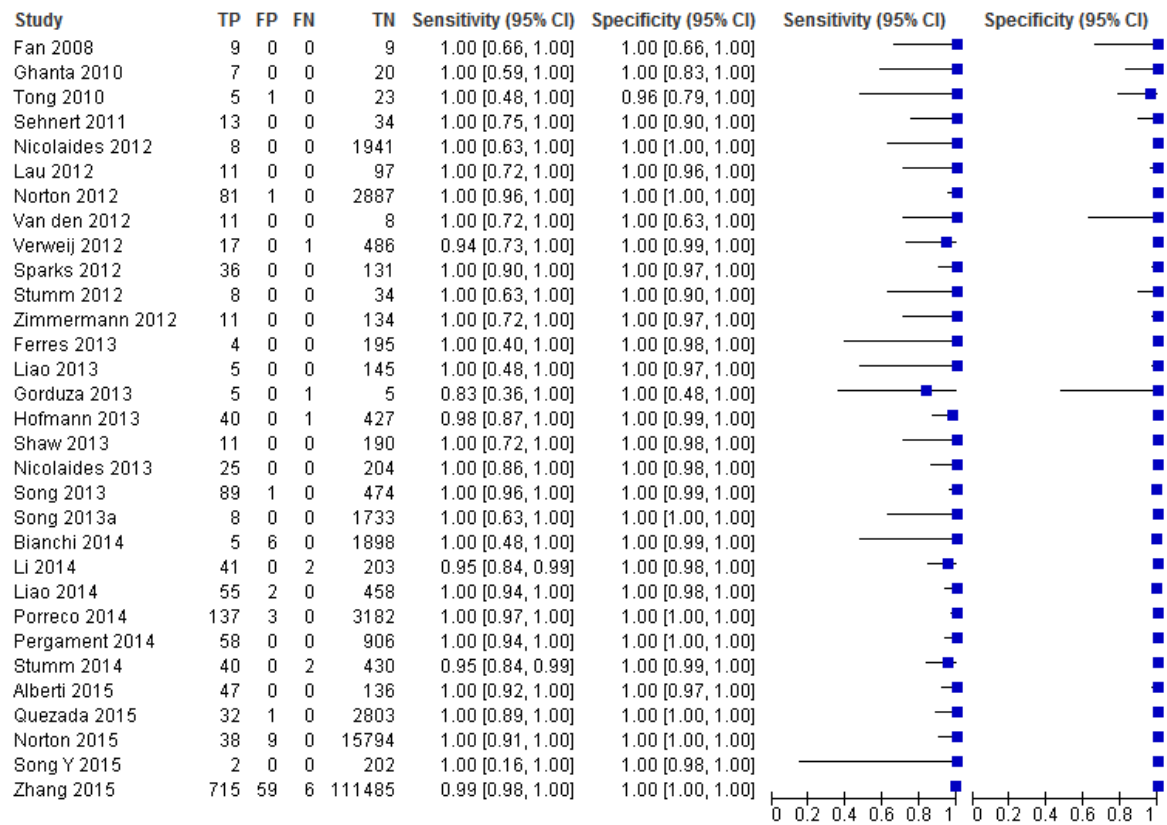


Figure 3B: Forest plot of studies testing Trisomy 18 using cell-free fetal DNA

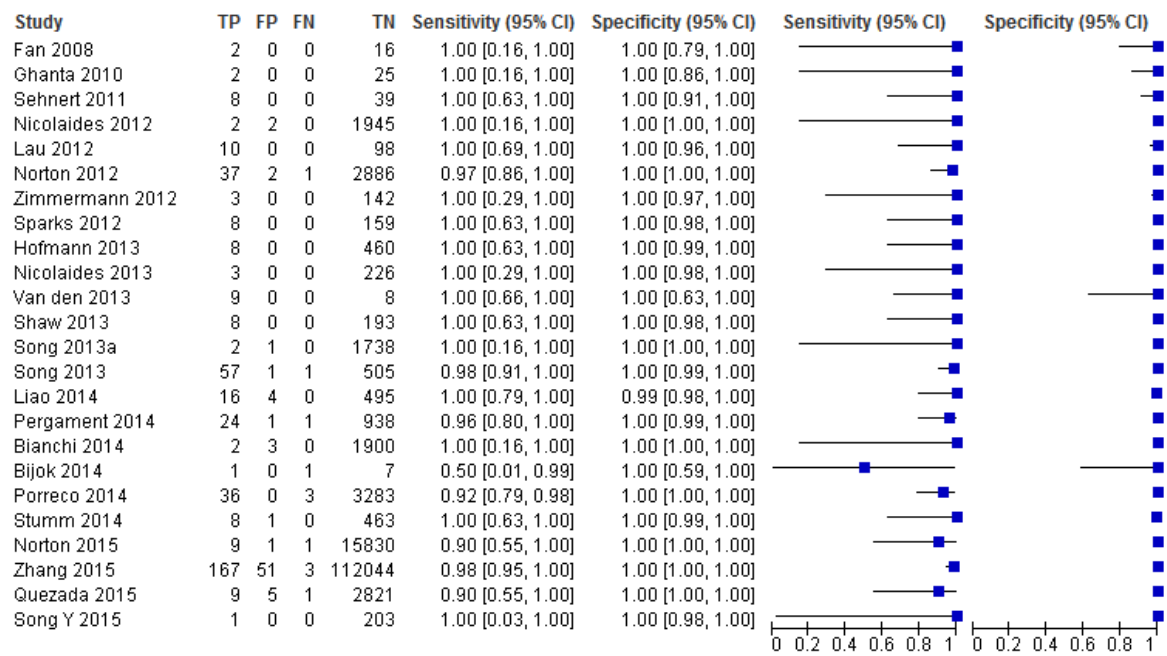


Figure 3C: Forest plot of studies testing Monosomy X using cell-free fetal DNA

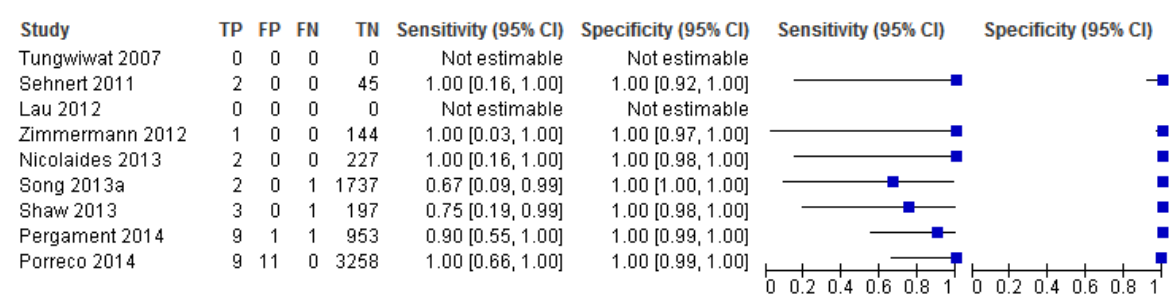


Figure 3D: Forest plot of studies testing Trisomy 13 using cell-free fetal DNA

